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<b>(54) Title:</b> MULTIFUNCTIONAL THROMBO-RESISTANT COATINGS AND METHODS OF MANUFACTURE		
<b>(57) Abstract</b> <p>The present invention is directed to multifunctional thrombo-resistant coatings for use with biomedical devices and implants, such as a coating which includes a siloxane surface onto which a plurality of amine functional groups have been bonded. Covalently bonded to the amine functional groups are a plurality of poly(ethylene oxide) chains, such that a single poly(ethylene oxide) chain is bonded to a single amine functional group. A plurality of different bioactive molecules, designed to counteract specific blood-material incompatibility reactions, are covalently bonded to poly(ethylene oxide) chains, such that a single bioactive molecule is coupled to a single polyethylene oxide chains. The methods of manufacturing the present invention include preparing a material having a siloxane surface onto which a plurality of amine functional groups have been bonded. This is achieved by plasma etching with ammonia gas or by plasma polymerization of a siloxane monomer in the presence of ammonia gas. The amine-containing siloxane surface is reacted with poly(ethylene oxide) chains terminated with functional groups capable of reacting with the amine groups on the siloxane surface. The material is then reacted with a plurality of different bioactive molecules which counteract the specific blood-material incompatibility reactions, such that a single bioactive molecule is coupled to a single poly(ethylene oxide) chain. The resulting siloxane surface contains a plurality of different bioactive molecules capable of reacting with blood components which come in proximity to the siloxane surface in order to resist blood-material incompatibility reactions.</p>		

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Multifunctional Thrombo-Resistant Coatings and Methods of  
Manufacture

BACKGROUND

1. The Field of the Invention

The invention relates to thrombo-resistant compositions for coating polymers and to the methods of manufacturing such coatings. More particularly, the present invention immobilizes on the surface of a gas permeable polymer, a wide range of bioactive substances which combat the various blood-material incompatibility reactions.

2. The Related Applications

This application is a continuation-in-part of copending application Serial No. 07/204,115, filed June 8, 1988, in the name of Gaylord Berry, J.D. Mortensen, and Larry D. Rigby and entitled "Apparatus and Method for In Vivo Extrapulmonary Blood Gas Exchange."

3. The Prior Art

Over the years, a large number of medical devices have been developed which contact blood. The degree of blood contact varies with the device and its use in the body. For instance, catheters may briefly contact the blood, while implants, such as heart valves and vascular grafts, may contact blood for a number of years. Regardless of the device, blood contact with foreign materials initiates the process of thrombosis, often followed by formation of thromboemboli.

Adsorption of proteins is one of the first events to occur when blood contacts a foreign surface. The compositions and conformation of adsorbed proteins influence subsequent cellular responses such as platelet adhesion, aggregation, secretion, complement activation, and ultimately, the formation of cross-linked fibrin and

1 thrombus. Thrombus formation is by far the most obvious  
and potentially debilitating response to foreign material  
in contact with blood.

5 The initial protein layer at the blood-material  
interface is subject to denaturation, replacement, and  
further reaction with blood components. During this phase  
of protein adsorption, adsorbed fibrinogen is converted to  
fibrin. Fibrin formation is accompanied by the adherence  
10 of platelets and possibly leucocytes. The platelets become  
activated and release the contents of their granules. This  
activates other platelets, thereby resulting in platelet  
aggregation.

A thrombus eventually forms from entrapment of  
erythrocytes (red blood cells) and other blood constituents  
15 in the growing fibrin network. Thrombus growth can  
eventually lead to partial or even total blockage of the  
device unless the thrombus is sheared off or otherwise  
released from the foreign surface as an embolus.  
Unfortunately, such emboli can be as dangerous as blockage  
20 of the device since emboli can travel through the  
bloodstream, lodge in vital organs, and cause infarction of  
tissues. Infarction of the heart, lungs, or brain, for  
example, can be fatal. Therefore, the degree to which the  
foreign material inhibits thrombus formation, embolization,  
25 and protein denaturation determines its usefulness as a  
biomaterial.

In the past, the thrombogenicity of biomedical  
implants has been treated by the administration of systemic  
anticoagulants, e.g., heparin and warfarin. However, long-  
30 term anticoagulation therapy is not advisable due to the  
risk of hazardous side effects. Moreover, overdose of  
anticoagulants may cause lethal side reactions, such as  
visceral or cerebral bleeding. For these reasons, there  
have been extensive efforts to develop materials which can

1 be used in biomedical devices or implants which can contact  
blood with minimal or no systemic anticoagulation therapy  
being necessary to avoid thrombus formation.

Many studies have attempted to produce a nonthrom-  
5 bogenic blood-contacting surface through immobilization of  
biologically active molecules onto the surface. Such  
bioactive molecules counteract various blood-material  
incompatibility reactions.

Surface modification of polymeric materials offers the  
10 advantage of optimizing the chemical nature of the  
blood/polymer interface while allowing a choice of the  
substrate to be based upon the necessary mechanical  
properties of the blood-contacting device.

The methods used to immobilize bioactive molecules  
15 onto blood-contacting surfaces fall into four general  
groups: physical adsorption, physical entrapment,  
electrostatic attraction, and covalent binding.

Surfaces incorporating bioactive molecules by physical  
adsorption or entrapment beneath the blood-contacting  
20 surface exhibit a significant degree of thrombo-resistance.  
However, depletion of the bioactive molecules into the  
blood environment causes the surface to rapidly lose its  
thrombo-resistant character. Entrained molecules diffuse  
to the surface which, along with physically adsorbed  
25 bioactives, are then "leached" from the surface into the  
blood plasma by mechanical and chemical mechanisms.

Similarly, electrostatically or ionically bound  
molecules are subject to partitioning and ion exchange  
between the blood-contacting surface and the electrolyte-  
30 rich plasma resulting in depletion. Covalently bound  
bioactive molecules resist depletion sufficiently to offer  
a potentially "long term" thrombo-resistant effect.

Numerous studies of covalent attachment of different  
biomolecules are available. These studies generally

1 involve the covalent attachment of a single bioactive  
molecule, usually heparin, designed to counteract one  
aspect of the blood-material incompatibility reactions.  
Most studies have focused on covalently binding heparin to  
5 a blood-contacting surface. Heparin is the most frequently  
prescribed anticoagulant in use today. It is a highly  
sulfonated mucopolysaccharide containing a number of  
charged functional groups. Heparin enhances the  
inactivation of thrombin by antithrombin III, thereby  
10 inhibiting the conversion of fibrinogen to fibrin.

Most prior attempts to covalently bind heparin to a  
blood-contacting surface have severely decreased the  
activity of heparin. For example, heparin coupled to a  
blood-contacting surface through one of its carboxyl groups  
15 loses up to 90% of its activity. Other systems, claiming  
covalent attachment of heparin, are actually heparin  
covalently bound to a coupling molecule which is  
subsequently ionically bound to the substrate.

Additional problems are encountered when the blood-  
20 contacting surface must also be gas permeable. Siloxane  
polymers are of particular interest in blood gas exchange  
devices because siloxane polymers not only possess certain  
inherent thrombo-resistant properties, but siloxane  
polymers also are gas permeable. However, siloxane  
25 polymers are relatively inert and pose a significant  
obstacle in modifying the surface in order to become more  
thrombo-resistant.

From the foregoing, it will be appreciated that what  
is needed in the art are multifunctional thrombo-resistant  
30 compositions and methods which counteract a wide range of  
blood material incompatibility reactions.

Additionally, it would be a significant advancement in  
the art to provide multifunctional thrombo-resistant  
compositions and methods which do not inhibit the gas

1 permeability of the blood-contacting surface.

It would be another advancement in the art to provide multifunctional thrombo-resistant compositions and methods in which the bioactive molecules are covalently bound to  
5 the blood-contacting surface, thereby eliminating elution of the bioactive molecules into the blood plasma.

It would be a further advancement in the art to provide multifunctional thrombo-resistant compositions and methods in which the bioactive molecules retain their  
10 activity after immobilization on the blood-contacting surface.

The foregoing, and other features and objects of the present invention, are realized in the multifunctional thrombo-resistant compositions and methods which are  
15 disclosed and claimed herein.

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1           BRIEF SUMMARY AND OBJECTS OF THE INVENTION

          The present invention is directed to multifunctional  
thrombo-resistant coatings for use with biomedical devices  
and implants. A variety of bioactive molecules which  
5 individually counteract specific blood-material  
incompatibility reactions are immobilized onto the  
polymeric surface of the device which is to contact the  
blood.

          Siloxane is the presently preferred substrate surface  
10 (that is, to which the multiple bioactive moleculars are  
bonded), because the substrate itself is initially  
relatively thrombo-resistant. Moreover, siloxane is gas  
permeable, thereby broadening the applications for the  
coatings of the present invention. Nevertheless, it will  
15 be appreciated from the specifications set forth below that  
other substrates are within the scope of the present  
invention.

          In order to overcome the inertness of the siloxane  
surface, functional groups, preferably amine groups, are  
20 introduced onto the siloxane surface. Two methods are  
currently preferred to introduce amine functionalities to  
the polymeric surface: (1) plasma etching with ammonia gas  
and (2) plasma polymerization with ammonia gas.

          In one currently preferred embodiment of the present  
25 invention, the amine groups on the siloxane surface are  
reacted with epoxide-, or isocyanate-terminated  
poly(ethylene oxide) (hereinafter referred to as "PEO").  
After such reaction occurs, the siloxane surface contains  
PEO chains coupled to the amine groups. The PEO spacer  
30 chains are presently preferred because the PEO tends to  
minimize protein adsorption.

          The unbound terminal end groups on the PEO chains  
readily react with the amine groups found in many bioactive  
molecules. Thus, various bioactive molecules may be  
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1 covalently bonded to one end of the PEO chains in the same  
way that the other end of the PEO chain is covalently  
bonded to the siloxane blood-contacting surface.

5 Since the bioactive molecules are spaced away from the  
siloxane surface at one end of a long PEO chain, the  
bioactive molecules possess an activity approaching the  
activity of the bioactive molecules in solution. Because  
of this mobility of the bioactive molecules near the blood-  
10 contacting surface of the polymer, the effectiveness of the  
bioactive molecules is substantially greater than the same  
bioactive molecules bound directly to the blood-contacting  
surface. At the same time, the serious risks associated  
with systemic anticoagulation therapy are avoided.

15 Some typical bioactive molecules which may be  
immobilized on a blood-contacting surface within the scope  
of the present invention include: heparin, ticlopidine,  
prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), urokinase, plasmin, and tissue  
plasminogen activator (TPA).

20 Heparin inhibits the blood incompatibility reaction  
resulting in clotting and thromboemboli formation by  
interacting with antithrombin III and thrombin to inhibit  
the conversion of fibrinogen to fibrin.

25 Ticlopidine and prostaglandin E<sub>1</sub> inhibit the activation  
of platelets either by minimizing aggregation or inhibiting  
activation and the release of the intracellular platelet  
activators. Each drug has a slightly different mode of  
action. Urokinase, plasmin, and TPA are all serine  
proteases which lyse formed protein deposits and networks.

30 All of the above blood incompatibility reactions are  
activated by the introduction of a foreign material into  
blood. Nonetheless, the present invention is unique,  
because it applies a multi-dimensional approach to  
combatting the problem of thrombus formation.

1        Systems which have only heparin counteract just the  
clotting mechanism involving the formation of fibrin.  
Other systems attempt to inhibit platelet activation or  
aggregation. In classical anticoagulant therapy, only one  
5 of the many blood-material incompatibility reactions is  
inhibited. The present invention is multifunctional  
because it is capable of inhibiting a wide range of the  
blood-material incompatibility reactions.

It is, therefore, an object of the present invention  
10 to provide multifunctional thrombo-resistant compositions  
and methods of manufacture which counteract a wide range of  
blood material incompatibility reactions.

Another important object of the present invention is  
to provide multifunctional thrombo-resistant compositions  
15 and methods which do not inhibit the gas permeability of  
the blood-contacting surface.

An additional important object of the present  
invention is to provide multifunctional thrombo-resistant  
compositions and methods in which the bioactive molecules  
20 are covalently bound to the blood-contacting surface,  
thereby eliminating elution of the bioactive molecules into  
the blood plasma.

Still another object of the present invention is to  
provide multifunctional thrombo-resistant compositions and  
25 methods in which the bioactive molecules retain their  
activity after immobilization onto the blood-contacting  
surface.

These and other objects and features of the present  
invention will become more fully apparent from the  
30 following description and appended claims, or may be  
learned by the practice of the invention.

1        DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

      The present invention provides a multifunctional thrombo-resistant coating for use with a blood-contacting surface of a medical device or implant. While it will  
5 immediately be appreciated that the present invention is applicable to a wide variety of medical device and implants, the coatings of the present invention are particularly suited for use with blood gas exchange devices. In any blood gas exchange device it is critical  
10 to both minimize thrombus and emboli formation, while at the same time preserving the gas exchange capabilities of the device.

      Accordingly, for purposes of illustration, the coatings of the present invention are discussed with  
15 respect to one such blood gas exchange device (as described in the above-identified copending patent application entitled "Apparatus and Method for In Vivo Extrapulmonary Blood Gas Exchange"); however, it is not intended that the invention is to be construed as limited for use on only  
20 such devices.

A. Multifunctional Bioactive Molecules

      To minimize the thrombo-resistant properties of any blood-contacting surface within the scope of the present  
25 invention, a wide variety of bioactive molecules which counteract specific blood-material incompatibility reactions are immobilized or linked to the blood-containing surface. It is an important feature of the present invention that a plurality of different bioactive molecules  
30 can be immobilized on the surface in order to inhibit a plurality of blood-material incompatibility reactions.

      These bioactive molecules inhibit blood material incompatibility reactions such as: coagulation and thrombus formation; platelet destruction, injury,  
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1 entrapment, and aggregation; complement activation; and  
protein adsorption. Table I provides a summary of the  
various bioactive molecules which may be used within the  
scope of the present invention to combat blood-material  
5 incompatibility reactions.

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TABLE I

	BLOOD INCOMPATIBILITY REACTION	BIOACTIVE SUBSTANCE	TYPE OF BIOACTIVITY
5	—		
	Extrinsic coagulation pathway activation to	Heparin	Interruption of the conversion of fibrinogen fibrin
10	—		
	Platelet destruction and injury, adhesion, and aggregation	Prostaglandin E <sub>1</sub> (PGE <sub>1</sub> )	Inhibits platelet shape change, platelet factor release, secretion and aggregation
15		Ticlopidine	Protects plate- lets and inhibits platelet aggregation
20	Fibrin Formation	Plasmin	Lyses fibrin
		Urokinase	Converts plasmin- ogen to plasmin, general proteo- lytic enzyme.
25		TPA	Activates plasminogen
	Protein adsorption	Poly(ethylene oxide)	Minimizes and prevents protein adsorption
30	Complement activation	FUT-175	Inhibits C1 <sup>r</sup> , C1 <sup>s</sup> , thrombin, and kallikrein
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1       The various bioactive molecules immobilized onto the  
surface give the blood-contacting surface a multifunctional  
thrombo-resistant coating. The term "thrombo-resistant" is  
generally used herein to generically represent the action  
5 of inhibiting the variety of blood incompatibility  
reactions discussed above. The surface is multifunctional  
because a plurality of different bioactive molecules are  
linked to the surface in a sufficient concentration to  
counteract a wide range of blood-material incompatibility  
10 reactions.

As mentioned above, an important feature of the  
present invention is the multifunctional inhibition of a  
plurality of blood incompatibility reactions. Hence, the  
present invention is in contrast to traditional techniques  
15 which deal with a single bioactive molecule and a single  
aspect of the blood-material incompatibility reactions.  
Thus, despite substantial surface contact with blood,  
thrombus formation on the surface of the medical device or  
implant (e.g., a blood gas exchange device) is  
20 inhibited/counteracted according to the compositions and  
methods within the scope of the present invention.

It will be appreciated that Table I lists only a few  
of the bioactive substances which inhibit the identified  
blood-material incompatibility reactions and that other  
25 bioactive substances may be used in accordance with the  
present invention to make a surface thrombo-resistant. As  
is discussed hereinafter, another important feature of the  
bioactive molecules used in the present invention is the  
availability of a primary amine (or other suitable  
30 functional groups) to react with the unbound functional end  
group on a molecule attached to the substrate surface.

1    B. Blood Gas Exchange Device

          The blood gas exchange devices to which the present invention is particularly applicable include both "sheet" membrane and tubular "membrane" oxygenators. Numerous  
5   oxygenators of these types are well known in the prior art.

          For purposes of illustration, one blood gas exchange device to which the present invention is applicable includes a dual lumen tube containing two coaxial lumens. The outer lumen opens into a proximal chamber to which the  
10   proximal ends of a plurality of elongated gas permeable tubes are attached. The inner lumen extends past the outer lumen and passes among the gas permeable tubes. Both the inner lumen and the distal ends of gas permeable tubes open into a distal chamber.

15       The device is inserted into the patient's venae cavae through an incision made in either the common femoral vein or the external iliac vein. The gas permeable tubes are crimped in order to maintain the tubes in a spaced relation one from another so that the blood may flow freely between  
20   and around the tubes, thereby enhancing the blood surface contact with the gas permeable tubes.

          One of either the inner or outer lumens is connected to a source of oxygen-rich gas. The other lumen is connected to an exhaust tube or other means for allowing  
25   the gas to flow out of the device. The oxygen-rich gas flows through the gas permeable tubes. As venous blood flows around the gas permeable tubes, oxygen passes from the tubes into the blood, thereby causing blood oxygenation, and carbon dioxide passes from the blood into  
30   the tubes and out of the body.

          One of the primary goals of a blood gas exchange device (whether or not it has the specific configuration discussed above) is to maximize the gas transfer surface area in contact with the blood. Unfortunately, as the  
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1 surface area of a foreign device in contact with blood increases, the risk of triggering a host of blood-material incompatibility reactions also increases.

Traditionally, as mentioned above, when a large  
5 quantity of blood contacts a foreign surface, systemic anticoagulants or thrombolytic agents are administered. Extreme care must be taken when administering any anticoagulants or thrombolytic agents to avoid the potential risk of serious hemorrhage both internally and  
10 externally. Thus, it is important that the blood-contacting surface of a blood gas exchange device is both gas permeable and thrombo-resistant. For these reasons, when the present invention is used with a blood gas exchange device, the blood-contacting surface is preferably  
15 constructed of a thin siloxane polymer.

C. Linking the Bioactive Molecules onto the Blood-Contacting Surface

For purposes of illustration, reference will be made  
20 to "linking" or "immobilizing" bioactive molecules on the blood-contacting substrate surface of a blood gas exchange device. It will be readily appreciated that the principles and teachings of the present invention are generally applicable to most other medical devices and implants which  
25 contact blood and have a problem with thrombus and emboli formation.

Moreover, it will be appreciated that the term "immobilized" is being used in the sense that the bioactive molecules are covalently linked or "tethered" to a specific  
30 portion of the polymer substrate vis-a-vis free floating in the blood. Therefore, even though the bioactive molecules may not be directly attached to the blood-contacting surface (as discussed in greater detail below), the bioactive molecules are closely associated to the surface

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1 through a linkage such that the blood cells contact the  
bioactive molecules as they come proximate to the blood-  
contacting surface.

5 Most of the bioactive molecules described above are  
capable of being immobilized to the blood-contacting  
surface of the blood gas exchange device through PEO  
coupling molecules. PEO is the preferred coupling  
molecule, because PEO itself functions to minimize protein  
adsorption. This property of PEO is believed to be due in  
10 part to PEO's unique hydrophobic and hydrophylic  
characteristics.

Because the blood-contacting surface of the blood-gas  
exchange device is preferably constructed of siloxane, the  
inherent inertness of the siloxane polymer minimizes  
15 thrombus formation. However, this same inherent inertness  
of the siloxane significantly complicates the method of  
immobilizing the bioactive molecules to the surface.

To overcome the inertness of the siloxane, functional  
groups are introduced on the siloxane surface. These  
20 functional groups provide distinct and predictable sites  
for reaction with PEO. The PEO chains are then coupled to  
the blood-contacting surface through the functional groups.  
In the currently preferred embodiment of the present  
invention, amine groups are introduced onto the siloxane  
25 surface.

#### 1. Introduction of Amine Groups by Plasma Etching.

One proposed method for introducing amine groups on  
the siloxane surface within the scope of the present  
30 invention involves plasma etching with ammonia gas. In the  
blood-gas exchange device of the present invention,  
microporous hollow fibers coated with a plasma-polymerized

1 siloxane are used as the substrate. These fibers are  
subjected to additional plasma exposure in the presence of  
ammonia gas.

5 The term "plasma" refers to a partially ionized gas  
which is in a non-equilibrium state. The electrons can  
react with gases or other materials present in the system  
producing a number of reactive particles and radiation such  
as cations, anions, free radicals, excited molecules,  
ultraviolet radiation, etc. By nature, plasma reactions  
10 are somewhat uncertain and unpredictable.

The pressure, temperature, gas flow rates, exposure  
time, power, and other parameters in a plasma process are  
highly interdependent and highly dependent upon the size  
and geometry of the plasma chamber. The power per unit  
15 area is an important parameter in reproducibly controlling  
the chemical structure of the resulting polymer. However,  
since plasma etching procedures and techniques are well  
known, a detailed discussion of each of the process  
parameters is not provided.

20 One plasma chamber used for plasma etching within the  
scope of the present invention has a volume of about 20,000  
 $\text{cm}^3$  and capacitively coupled plate electrodes. The plasma  
chamber was obtained commercially from Plasma Science  
(Belmont, California), and modified by the inventors by  
25 removing the two lower electrode plates so that the chamber  
would accommodate a smaller cylindrical plasma chamber.  
The siloxane plasma-coated fibers, having a surface area of  
about 2,100  $\text{cm}^2$ , are exposed to ammonia having a flow rate  
in the range of from about 100 micromoles per second to  
30 about 300 micromoles per second, at an absolute pressure in  
the range from about 100 millitorr to about 200 mtorr. The  
exposure time ranges from about thirty (30) seconds to  
about three (3) minutes. The currently preferred exposure  
time is in the range from about 60 seconds to about 120

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1 seconds. A radio frequency of 13.56 MHz in the range from  
about 20 watts to about 250 watts generates sufficient  
energy to break the molecular bonds of both the ammonia gas  
and the siloxane surface.

5 Another plasma chamber used for plasma etching has a  
volume of about 28.7 cm<sup>3</sup> and capacitively coupled copper  
collar electrodes located outside the tube. The chamber is  
cylindrical, having a diameter of about one centimeter and  
a length of about 25 centimeters. The siloxane plasma-  
10 coated fibers, having a surface area of about 3.03 cm<sup>2</sup> are  
exposed to ammonia having a flow rate in the range from  
about 10 micromoles per second to about 120 micromoles per  
second, at an absolute pressure in the range from about 100  
mtorr to about 200 mtorr. The exposure time ranges from  
15 about 30 seconds to about 120 seconds. A radio frequency  
of 13.56 MHz in the range from about 20 watts to about 150  
watts generates sufficient energy to break the molecular  
bonds of both the ammonia gas and the siloxane surface.

20 It will be appreciated by those skilled in the art  
that in a differently configured plasma chamber, the  
ammonia flow rate, power, chamber pressure, and exposure  
time may be outside the ranges of that set forth for the  
embodiment discussed above. Nevertheless, current  
experimental testing suggests that the power should relate  
25 to the monomer or gas flow rate such that  $W/FM$  is in the  
range from 30-50 megajoules/Kg, where  $W$  is the discharge  
power in joules per second,  $F$  is the mass flow rate in  
moles per second, and  $M$  is the molecular weight of a gas  
(g/mole). However, this value ( $W/FM$ ) does not take into  
30 consideration the power density which is determined by the  
volume of the plasma. Because the minimum wattage  
necessary for the plasma polymer of a given monomer differs  
significantly from that of another monomer at a given  
pressure, it becomes immediately obvious that  $W$ , wattage  
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1 per square centimeter, or current density alone is not  
sufficient to describe the conditions of plasma  
polymerization. Hence, the flow rate, power, and pressure  
may well be outside of the ranges given.

5 In light of these stoichiometric relationships, those  
skilled in the art can readily determine relationships  
between the flow rate, the pressure, and the exposure times  
of the siloxane surface to the ammonia.

Plasma may be generated by a number of methods  
10 including combustion, flames, electric discharge,  
controlled nuclear reactions and shocks. The most obvious  
and commonly used is the electric discharge. Radio  
frequency (RF) or microwave discharge are mainly used for  
polymerization reactions. For the commercial RF  
15 generators, the frequency is dictated by the Federal  
Communications Commission and is set at 13.56 MHz.

Ammonia derivatives, existing as free radicals and  
ions react with each other and with the siloxane surface,  
thereby introducing amine functionalities onto the siloxane  
20 surface. Analysis by electron spectroscopy for chemical  
analysis ("ESCA") establishes that nitrogen in the form of  
amine functionalities can be introduced onto the surface on  
the order of from about two (2) to about eight (8) total  
atomic percent. ESCA measurements of about three total  
25 atomic percent have been found to result in a satisfactory  
end product. Other polymers not as inert as siloxanes are  
capable of incorporating much higher amounts of nitrogen.

It should be noted that ESCA analyzes only the top 50-  
100 angstroms of a surface. Analysis of bulk structure  
30 below the sampling depth is not possible with ESCA. In  
addition, the atomic percent reported by ESCA is for the  
entire volume analyzed (i.e., the top 50-100 angstroms).  
Thus, 3% nitrogen does not correspond with 3% of the  
surface atoms being nitrogen. This is because the nitrogen

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1 atoms would be found only on the surface and atoms (i.e. carbon/silicone) from below the surface are also detected.

Nevertheless, ESCA does establish the existence of significant amounts of nitrogen at or near the surface. Moreover, analysis of percent nitrogen provides a valuable approximation for the number of free amines on the surface. The quantity of amines bound to the surface directly affects the coupling efficiency of the PEO or bioactive molecules. Thus, the more amine groups, the more PEO coupling sites.

From the foregoing, it will be appreciated that the parameters associated with ammonia etching are highly interdependent and dependent upon the specific plasma chamber. The following examples illustrate this interdependence. One skilled in the art would appreciate that the parameters described in the following examples can be modified when using a different sized plasma chamber.

#### EXAMPLE 1

20 Amine groups were introduced onto the surface of a siloxane-coated hollow fiber within the scope of the present invention by plasma etching in the presence of ammonia. Celanese X20-240 microporous hollow fibers were used as the substrate. The fibers were coated with plasma-polymerized siloxane.

The fibers were subjected to additional plasma exposure in the presence of ammonia gas by passing the fibers through a cylindrical plasma chamber one centimeter in diameter and approximately 25 centimeters long with two copper collar electrodes capacitively coupled to the chamber. The surface area of the fibers was about 3.0 cm<sup>2</sup>. Ammonia gas was introduced into the plasma chamber at a flow rate of 30 micromoles per second at 110 mtorr absolute

1 pressure. The fibers were exposed to 45 watts at a radio  
frequency of 13.56 MHz for 60 seconds.

According to ESCA analysis, nitrogen in the form of  
amine functionalities was introduced onto the surface on  
5 the order of three total atomic percent. As discussed  
hereinafter, this amount of nitrogen provides sufficient  
amine reaction sites for attachment of the PEO and the  
multifunctional bioactive molecules.

10

#### EXAMPLE 2

Amine groups were introduced onto the surface of a  
siloxane-coated hollow fibers according to the procedure of  
Example 1, except that the ammonia gas was introduced into  
the plasma chamber at a flow rate of 120 micromoles per  
15 second at 110 mtorr absolute pressure. The fibers, having  
a surface area of about 3.0 cm<sup>2</sup>, were exposed to 60 watts at  
a radio frequency of 13.56 MHz for 30 seconds.

Utilizing the procedures of Example 2, nitrogen in the  
form of amine functionalities was introduced onto the  
20 surface as analyzed by ESCA on the order of three total  
atomic percent. While the flow rate of the ammonia gas in  
the plasma chamber was four times greater than that of  
Example 1, no significant increase in the amount of amine  
functionalities on the siloxane surface were observed.

25

#### EXAMPLE 3

Amine groups were introduced onto the surface of  
siloxane-coated hollow fibers according to the procedure of  
Example 1, except that the fibers were exposed to 20 watts  
30 at a radio frequency of 13.56 MHz for two minutes.

Utilizing the procedures of Example 3, nitrogen in the  
form of amine functionalities were introduced onto the  
surface as analyzed by ESCA on the order of two total  
atomic percent. While the fibers of Example 3 were exposed

35

1 to only 40% of the power used on the fibers of Example 1,  
there was only a slight decrease in the amount of amine  
functionalities on the siloxane surface.

5 EXAMPLE 4

Amine groups were introduced onto the surface of  
siloxane-coated hollow fibers according to the procedure of  
Example 1, except that the ammonia gas was introduced into  
10 the plasma chamber at a flow rate of 120 micromoles per  
second at 110 mtorr absolute pressure. The fibers were  
exposed to 20 watts at a radio frequency of 13.56 MHz for  
30 seconds.

Utilizing the procedures of Example 4, nitrogen in the  
form of amine functionalities was introduced onto the  
15 surface as analyzed by ESCA in less than two total atomic  
percent. Fiber exposure to 20 watts for 30 seconds was  
insufficient for adequate nitrogen incorporation.

EXAMPLE 5

20 Amine groups were introduced onto the surface of a  
siloxane substrate within the scope of the present  
invention by plasma etching in the presence of ammonia.  
The dimensions of the plasma chamber were fifteen inches  
long, twelve inches wide and five inches high. The  
25 electrodes were in the form of two parallel plates  
capacitively coupled in the chamber. The siloxane-coated  
substrate is comprised of a siloxane coating on a polymeric  
surface. The siloxane surface, with a surface area of  
2,100 cm<sup>2</sup> is subjected to additional plasma exposure by  
30 introducing ammonia gas into the plasma chamber at the flow  
rate of 288 micromoles per second at 180 mtorr absolute  
pressure. The siloxane surface is exposed to 186 watts at  
a radio frequency of 13.56 MHz for two minutes.

35

1 According to ESCA analysis, nitrogen species are  
introduced onto the surface on the order of 3.5 total  
atomic percent.

5 EXAMPLE 6

Amine groups were introduced on the surface of a  
siloxane-coated polyethylene substrate according to the  
procedure of Example 5, except that the siloxane-coated  
substrate is exposed to 150 watts at a radio frequency of  
10 13.56 MHz for a period of 90 seconds.

Utilizing the procedures of Example 6, nitrogen  
containing functionalities are introduced onto the  
siloxane-coated surface as analyzed by ESCA on the order of  
four total atomic percent.

15

EXAMPLE 7

Amine groups were introduced onto the surface of a  
siloxane substrate within the scope of the present  
invention by plasma etching in the presence of ammonia.  
20 The siloxane substrate, comprised of a siloxane coated  
glass slide, was subjected to additional plasma exposure by  
placing the substrate into the cylindrical plasma chamber  
one centimeter in diameter and approximately 25 centimeters  
long. Ammonia gas was introduced into the plasma chamber  
25 at a flow rate of 12 micromoles per second. The pressure  
within the chamber was maintained at 180 mtorr absolute  
pressure. The siloxane substrate was exposed to 100 watts  
at a radio frequency of 13.56 MHz for ten minutes.

According to ESCA analysis, nitrogen in the form of  
30 amine functionalities was introduced onto the surface on  
the order of eight total atomic percent. The higher  
incorporation of nitrogen was attributed to a different  
type of siloxane substrate and an increase in power and

35



1 exposure time made possible because the glass substrate is  
nonfragile and can withstand prolonged plasma exposure.

#### 5 EXAMPLE 8

5 Amine groups were introduced onto the surface of a  
siloxane substrate within the scope of the present  
invention by plasma etching in the presence of ammonia.  
The siloxane substrate, comprised of a methyl vinyl  
10 siloxane coated onto a glass slide, was subjected to plasma  
exposure by placing the substrate into the cylindrical  
plasma chamber one centimeter in diameter and approximately  
25 centimeters inches long. Ammonia gas was introduced  
into the plasma chamber at a flow rate of 12 micromoles per  
15 second. The pressure within the chamber was maintained at  
180 mtorr absolute pressure. The siloxane substrate was  
exposed to 50 watts at a radio frequency of 13.56 MHz for  
15 minutes.

According to ESCA analysis, nitrogen in the form of  
amine functionalities was introduced onto the surface on  
20 the order of 22 total atomic percent. The higher  
incorporation of nitrogen was attributed to a different  
type of siloxane substrate and an increase in power and  
exposure time made possible because the silicone-coated  
glass substrate was nonfragile and can withstand prolonged  
25 plasma exposure.

#### 2. Introduction of Amine Groups by Plasma Polymerization.

Another method for introducing the amine  
functionalities onto the blood-contacting surface of the  
30 siloxane polymer is to introduce the amine groups during  
the siloxane polymerization itself. This process, known as  
plasma polymerization or glow discharge polymerization, is  
achieved by introducing a siloxane monomer vapor and  
ammonia gas simultaneously in the presence of the plasma.  
35

1 The same type of tubular chamber used for plasma etching  
(Examples 1-4) may be used for plasma polymerization.

Two opposing processes occur simultaneously during  
plasma polymerization: (1) polymer formation which leads  
5 to deposition of a material and (2) ablation which leads to  
removal of material. Generally, at very low flow rates  
there is little polymer deposition and the deposition rate  
decreases with increasing discharge wattage. At higher  
flow rates, the deposition increases (linearly), but  
10 reaches a maximum with increasing discharge wattage and  
then ablation becomes more predominant.

The amount and relative position of polymer deposition  
is influenced by three geometric factors: (1) location of  
electric energy input; (2) monomer flow; and (3) substrate  
15 position within the reactor relative to the glow region.  
These factors are only important in batch polymerization  
processes. In the case of hollow fibers, which are pulled  
continuously through the plasma chamber, the influence of  
the substrate position is averaged over the length of the  
20 fibers.

The population of energetic species that contribute to  
the direct formation of plasma polymer is not directly or  
uniquely related to the power input into the system. The  
intensity of a non-polymer forming plasma (i.e., plasma  
25 etching) is dependent on the combined factors of pressure  
and discharge power as well as on other factors of the  
discharge system such as distance between electrodes,  
surface area of electrodes, and total volume of the  
reactor.

30 Various parameters have been used to describe the  
energy input of plasma polymerization such as current  
density, current and voltage, or wattage. These parameters  
may have varying degrees of applicability to an inductively  
coupled RF discharge system. However, such parameters are  
35

1 insufficient to describe the change in total volume of  
plasma and the plasma polymerization that takes place in  
the volume, although certain correlations can be found  
between the deposition rates and these parameters, but only  
5 for a given set of experimental conditions.

An important feature of the present invention,  
particularly for use with a blood oxygenator, is the  
creation of a smooth, continuous (pin-hole free) thin  
coating over the pores of the hollow fiber. The thickness  
10 of this coating can be determined gravimetrically, and the  
continuity of the coating can be determined by the  
permeability. These factors, along with the chemical  
composition (i.e., carbon, silicone, oxygen, nitrogen  
percentages, determined by ESCA) are some of the values  
15 which change as plasma parameters are modified.

The chemical composition of the plasma coating affects  
the gas permeability. For example, as the cross-link  
density increases, the permeability decreases. Factors  
which affect the cross-link density include: pressure,  
20 power, flow rate, and position within the reactor. Gas  
permeability is also influenced by the plasma deposition  
thickness and the completeness of coverage of the pores.

In order to achieve plasma polymerization, the  
siloxane monomer and ammonia gas in a concentration ratio  
25 in the range from about 1:10 to about 10:1 (and preferably  
about 3:1, siloxane monomer to ammonia) and at an absolute  
pressure in the range from about 100 to about 200 mtorr,  
are introduced into the plasma chamber. One presently  
preferred siloxane monomer is tetramethyldisiloxane,  
30 commonly known as "TMDS." Other suitable siloxane monomers  
include hexamethyldisiloxane, octamethyltrisiloxane,  
hexamethylcyclotrisiloxane and octamethyl-  
cyclotetrasiloxane.

1        In one embodiment within the scope of the present  
invention, siloxane monomer with a flow rate in the range  
from about ten micromoles per second to about 30 micromoles  
per second and ammonia gas with a flow rate in the range  
5       from about ten micromoles per second to about 30 micromoles  
per second are introduced into the plasma chamber.  
Capacitatively coupled power in the range from about 45 to  
about 60 watts at a frequency of 13.56 MHz of a radio  
frequency generator is applied to create the plasma.

10        The hollow fibers are pulled through the plasma zone  
such that the total residence time in the plasma is in the  
range from about 30 seconds to about 70 seconds. Nitrogen  
in the form of amine functionalities is introduced onto the  
siloxane surface as analyzed by ESCA on the order of about  
15    six (6) to eight (8) total atomic percent.

Care should be taken when polymerizing siloxane in the presence of ammonia that too much ammonia is not incorporated into the resulting polymer coating. It would be expected that as the concentration of nitrogen increases, the gas permeability of the polymer decreases. Accordingly, the percentage of the nitrogen functionalities in the siloxane coating should not exceed about eight (8) total atomic percent; otherwise, the gas permeability may be significantly decreased.

25     As with plasma etching, power distribution in the plasma chamber used in this plasma polymerization process can be determinative of the process parameters used. The following examples illustrate this interdependence.

30 EXAMPLE 9

The surface of polypropylene hollow fibers was coated with a nitrogen-containing siloxane plasma polymer within the scope of the present invention by plasma polymerization in the presence of ammonia. Polypropylene microporous

1 hollow fibers were used as the substrate.

The fibers were subjected to plasma polymerization by passing the fibers through a cylindrical plasma chamber one centimeter in diameter and approximately 25 centimeters  
5 long. Nine micromoles per second tetramethyldisiloxane along with three micromoles per second ammonia gas were introduced into the plasma chamber.

The plasma was struck at 150 watts using a radio frequency generator at a frequency of 13.56 MHz and then  
10 reduced to 45 watts following striking. A higher power is necessary to "strike" the plasma in order to initiate bond cleavage. Thereafter, the power is reduced before introducing the fibers into the plasma chamber, otherwise the high power could destroy the fragile fibers.

15 The pressure in the plasma chamber was maintained at 110 mtorr absolute pressure by use of a vacuum throttling valve. The fibers were pulled through the plasma zone such that the total residence time in the plasma was 60 seconds.

According to ESCA analysis, nitrogen containing  
20 functionalities were introduced and detected on the surface of the fiber on the order of six total atomic percent. Presumably amine functionalities were incorporated into the bulk of the fiber as well. Scanning Electron Microscope analysis (SEM) demonstrated the thickness of the coating to  
25 be less than 1 micron.

#### EXAMPLE 10

The surface of polypropylene hollow fibers was coated  
30 with a nitrogen-containing siloxane plasma polymer

35

1 according to the procedure of Example 9, except that the  
residence time of the fibers in the plasma was 70 seconds.

Utilizing the procedures of Example 10, nitrogen-  
5 containing functionalities were introduced throughout the  
bulk of the polymer with a surface concentration on the  
order of six total atomic percent as analyzed by ESCA.  
Gravimetric analysis of one meter of the fiber showed a  
gain of 0.7 milligrams. This indicated a coating of less  
10 than 1 micron.

#### EXAMPLE 11

The surface of polypropylene hollow fibers was coated  
with a nitrogen-containing siloxane plasma polymer  
15 according to the procedure of Example 9, except that the  
power was reduced to sixty (60) watts following striking.

Utilizing the procedures of Example 11 amine  
functionalities were introduced throughout the bulk of the  
polymer as analyzed by ESCA on the order of six total  
20 atomic percent. Gravimetric analysis of one meter of fiber  
indicated the thickness of the coating was 1.0 micron.

#### EXAMPLE 12

The surface of polypropylene hollow fibers was coated  
25 with a nitrogen-containing siloxane plasma polymer within  
the scope of the present invention by plasma polymerization  
in the presence of ammonia. Celanese X20-240 microporous  
hollow fibers were used as the substrate.

The substrate is subjected to plasma polymerization by  
30 passing the substrate through a cylindrical plasma chamber  
1 centimeter in diameter and 25 centimeters long with two  
sets of capacitively coupled electrodes (*i.e.*, two hot and  
two ground). Nine micromoles per second of TMDS along with  
three micromoles per second of ammonia gas are introduced

35

1 into the plasma chamber. The plasma is struck at 150 watts  
at a radio frequency of 13.56 MHz and then reduced to 60  
watts after striking.

5 The pressure in the plasma chamber was maintained at  
110 mtorr absolute pressure by use of a vacuum throttling  
valve. The total residence time of the siloxane-coated  
substrate in the plasma was 60 seconds.

ESCA analysis indicated approximately 6% nitrogen on  
the surface. Gravimetric analysis determined the coating  
10 to be approximately 1.5 microns thick.

#### EXAMPLE 13

The surface of polypropylene hollow fibers was coated  
with a nitrogen-containing siloxane plasma polymer  
according to the procedure of Example 12, except that the  
15 pressure of the chamber was maintained at 180 mtorr.

Utilizing the procedures of Example 13, it was  
determined that melting of the fiber had occurred.

#### EXAMPLE 14

20 A siloxane coating containing nitrogen, hydroxyl and  
carbonyl functionalities was coated onto a glass substrate  
within the scope of the present invention by plasma  
polymerization in the presence of ammonia and water vapor.  
The glass substrate was subjected to plasma polymerization  
25 in the cylindrical plasma chamber described in Example 12  
except that water vapor was introduced along with the  
ammonia and monomer.

The water vapor flow rate was approximately three  
micromoles per second. The substrate was exposed to 60  
30 watts for a period of four minutes. Analysis of the  
coating by ESCA determined that 7.5% carbonyl, 15% hydroxyl  
and 4% amine functionalities were present.

1

EXAMPLES 15-18

In Examples 15-18, the surface of polypropylene hollow fibers was coated with a siloxane plasma polymer according to the procedure of Example 11, except that the ratio of TMDS to ammonia was varied from 10:1 to 1:10 in a constant molar gas flow rate of 12 micromoles per second.

The chemical composition of the resulting siloxane plasma polymers, as analyzed by ESCA, are set forth below:

10

TABLE II

Example	TMDS:Ammonia	C%	O%	Si%	N%
15	10:1	47	16	37	0
16	3:1	45	21	38	6
17	1:1	48	24	30	4
18	1:10	43	24	32	<1

These results indicate that a ratio of TMDS to ammonia of about 3:1 produces a siloxane plasma polymer with a high percent nitrogen incorporation.

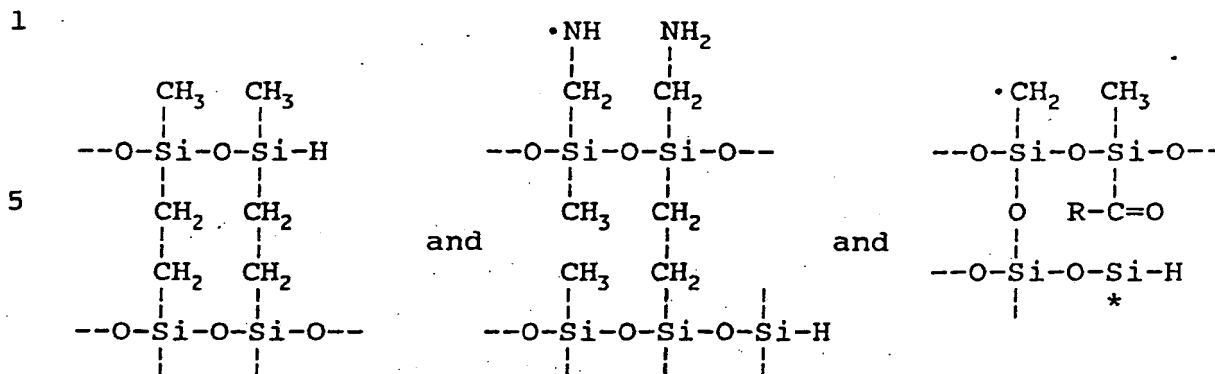
20

3. Amine Functionalities on the Siloxane Surface.

Both ammonia etching and plasma polymerization with ammonia result in amine incorporation into or onto the siloxane polymer. ESCA analysis of the resulting surface demonstrates the existence of Si-H bonds, C-N bonds, amine (NH<sub>2</sub>) groups, and carbonyl (C=O) groups. In addition, the surface likely includes reactive radicals (e.g., •CH<sub>2</sub> and •). While the exact surface structure resulting from these reaction processes is not known, the resulting surface structure is believed to be a combination of a number of possible bond and group configurations including:

35





R may be H or OH.

The degree of cross-linking (*i.e.*, the number of bonds formed from methyl radicals on adjacent polymer chains reacting together to form an ethylene unit between chains) is totally dependent upon the reaction parameters. Any polymerization performed using plasma results in a "plasma polymer." The structure of a plasma polymer is significantly different from those resulting from other known polymerization mechanisms; these plasma polymers are by nature "ill-defined."

It will be appreciated that an important aspect of the present invention is the incorporation of amine functionalities (which are available for reaction with PEO) on the blood-contacting surface. Hence, other plasma reaction processes which introduce amine onto the surface are useful as a part of the present invention.

For example, another possible process for introducing amine functionalities on the blood-contacting surface would be to coat the surface with siloxane monomer in the plasma, and then introduce another polymerizable gas which contains amine groups. One potentially suitable amine-containing polymerizable gas is allylamine.

1       The allylamine may be introduced while the surface is  
in the plasma or shortly after the plasma has been turned  
off. Such polymerization processes could result in an  
extremely thin polymer layer, probably only a few atomic  
5 layers thick on top of the siloxane, with a high percentage  
of primary amine groups. Theoretical calculations suggest  
that nitrogen containing functional groups could be  
incorporated onto the siloxane on the order of about twenty  
atomic percent.

10       Such a thin polymer layer should not adversely affect  
the overall gas permeability of the siloxane or its other  
mechanical properties. However, if the allylamine were  
polymerized to form more than just a few atomic layers, the  
gas permeability of the siloxane substrate might be  
15 significantly reduced. Since allylamine polymerization  
tends to preserve the amine groups rather than forming  
ammonia byproducts, an allylamine plasma polymerization has  
the potential of introducing a significantly higher  
percentage of potentially reactive amine groups on the  
20 siloxane surface.

In addition, depending on the type of siloxane monomer  
used to form the siloxane surface, nitrogen gas is a  
suitable alternative to ammonia gas in both the plasma  
etching and plasma polymerization processes described  
25 above. Nitrogen gas initially introduces both amine groups  
and nitrogen radicals onto the siloxane surface, but upon  
exposure to water vapor, the nitrogen radicals quickly  
quench to form amine groups. Because nitrogen is less  
expensive than ammonia, the use of nitrogen gas can  
30 significantly reduce the costs associated with the plasma  
process described above.

Although the foregoing discussion has focused on the  
incorporation of amine groups onto the siloxane surface, it  
will be appreciated that the principles within the scope of  
35

1 the present invention may be readily adapted to incorporate  
other reactive functional groups onto the siloxane surface.

5 Thus, an important aspect of the invention is the  
incorporation of any reactive functional group such as  
hydroxyl, carbonyl, or carboxylic groups onto the siloxane  
surface. These functional groups would provide a chemical  
"handle" on the otherwise inert siloxane surface to which  
PEO and bioactive molecules may be bound.

10 In this regard, other gases and monomers may be used  
during the plasma etching or plasma polymerization  
processes to introduce reactive functional groups. As  
illustrated in Example 14, above, the introduction of water  
vapor during the plasma polymerization process has been  
15 found to introduce carbonyl and hydroxyl functionalities  
onto the siloxane surface, as well as amine groups.

It has been found that plasma etching with argon gas  
or oxygen gas causes destruction of the hollow fibers (as  
measured by decrease in tensile strength) in less than one  
20 minute of exposure. On the other hand, similar exposure to  
ammonia gas did not destroy the hollow fibers. This is one  
reason why ammonia is currently preferred for plasma  
etching. Nevertheless, if the substrate is not fragile  
like the hollow fibers, then argon and oxygen plasmas may  
25 be used to introduce reactive functional groups onto the  
siloxane surface.

The surfaces which emerge from the plasma in any of  
the processes discussed above are highly reactive. While  
exact molecular analysis is difficult, the surfaces likely  
30 contain some radicals which are available for reacting with  
almost any species containing double bonds which come into  
contact with the siloxane surface.

1        4. Reaction of Amine Functionalities with PEO.

         Immediately upon removal from the plasma, the surfaces  
of the hollow fibers may be reacted with the terminal end  
groups of unbranched PEO. The PEO functions as an extended  
5       flexible spacer to tether bioactive molecules away from,  
but in close proximity to, the siloxane surface, thereby  
avoiding problems of steric hindrance of adjacent bioactive  
molecules which may then be coupled to the siloxane  
surface. Moreover, as discussed above, the PEO itself also  
10       assists in minimizing protein adsorption on the siloxane  
surface.

         A PEO solution is prepared by dissolving poly(ethylene  
oxide) bis(glycidyl ether) (commonly known as "PEO  
15       diglycidyl ether," or "polyoxyethylene diglycidyl ether")  
into a solution containing formamide and water. The  
concentration of formamide in water is in the range from  
about 25% to about 35% (preferably about 30%). The PEO  
must be in excess to minimize "looping" of the PEO by both  
20       reactive ends coupling to the amine groups on the surface.  
Typical PEO concentrations are in the range from about 5%  
to about 36%, and preferably about 9% to about 18%.

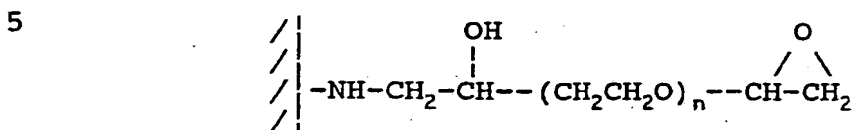
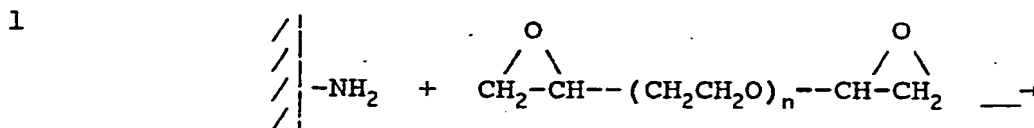
         Poly(ethylene oxide) bis(glycidyl ether) of any  
molecular weight may be used. However, for maximum protein  
resistance, the range should be from about 1500 to about  
25       6000 and preferably in the range from about 3000 to about  
4000. It has been found that PEO within this molecular  
weight range minimizes the protein adsorption and maximizes  
repulsion of platelets from the surface. There is a  
30       balance between chain length and stability as well. Longer  
chains are more susceptible to chain scission. Shorter PEO  
chains are less flexible which reduces their protein-  
resistant properties.

1 Many terminal reactive groups on PEO may be used  
depending upon the functionality on the siloxane to which  
coupling is desired. In the case of amine groups on the  
siloxane surface, suitable terminal groups include epoxides  
5 or isocyanates. In the case of carbonyl groups on the  
siloxane surface, amine terminated PEO would be  
appropriate. In any event, only those PEO chains with two  
reactive functional groups would be available for coupling  
to a surface and to a bioactive molecule.

10 In the case of epoxide-terminated PEO, the percent  
epoxide within the PEO varies depending upon the  
manufacturer and can vary from about 10% to greater than  
75% epoxide. The percentage epoxide directly affects the  
coupling efficiency. Therefore, if 100% of all PEO chains  
15 contain terminal epoxide groups, theoretically all could  
bind not only to the surface but also be available for  
binding bioactive molecules.

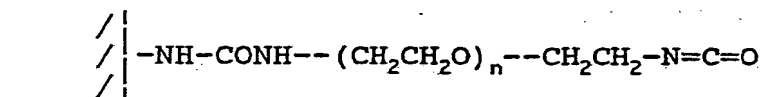
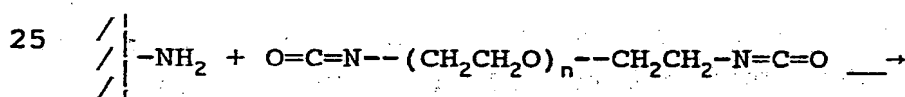
The plasma-coated fibers of the blood gas exchange  
device are allowed to sit in the PEO solution, without  
20 agitation, for about ten hours. It has been found that the  
amount of PEO coupling (as determined by ESCA) does not  
significantly increase after twelve (12) hours. In  
addition, increasing the concentration of PEO (to about 36  
weight percent in the solvent) does not significantly  
25 increase the amount of coupling over the same time  
interval. The temperature of the PEO solution is  
preferably maintained at ambient temperature, in the range  
from about 20°C to about 30°C.

After removal from the PEO solution, the coated hollow  
30 fibers are rinsed with purified water to remove any unbound  
PEO. The epoxide groups located at the terminal ends of  
the PEO chains have reacted with the amine groups located  
on the siloxane surface as shown below:



Due to the large excess of PEO used and reaction conditions, only one end of the PEO chain is bound to an amine group on the siloxane surface. As a result, each PEO chain contains an unreacted epoxide group at its unbound end. In addition, any carbon radicals ( $\cdot\text{CH}_2$ ) remaining on the surface following plasma polymerization would not be expected to react with the epoxide groups and would continue to be reactive.

Alternatively, it has been found that the PEO chains may also be suitably terminated with isocyanate functionalities. The amine groups located on the siloxane surface react with the isocyanate in much the same way as the amine nitrogen reacts with the epoxide terminated PEO as shown below.



The epoxide effectively reacts with the electron-rich amine nitrogen because epoxide is a highly strained three-member ring. It also contains an electron depleted carbon atom. The epoxide efficiency is due mainly to the strained

1 ring. The isocyanate reacts well with the amine nitrogen  
because the isocyanate carbon is accessible and electron  
depleted.

5 It will be appreciated that the PEO chains may be  
suitably terminated with other functional groups such as  
imidazole carbonyl. The important considerations in  
selecting a suitable functional group are its attachability  
to PEO and its activity with amines. Nevertheless, the  
epoxide and isocyanate terminated PEO have been found to  
10 produce a satisfactory product without elaborate and  
complex reaction conditions.

Despite the process used to incorporate the amine  
functionalities onto the surface of the polymeric  
substrate, the PEO can readily react with the amine groups  
15 to attach the PEO to the siloxane (or other suitable  
polymer) substrate, as shown in the following examples.

#### EXAMPLE 19

20 Siloxane-coated hollow fibers on which amine  
functionalities have been incorporated onto the siloxane  
surface according to the procedures of Example 1 were  
reacted with a solution containing poly(ethylene oxide)  
bis(glycidyl ether). This PEO solution was prepared by  
dissolving eighteen grams of PEO bis(glycidyl ether) having  
25 average molecular weight of 3,500 in 100 ml of a solvent  
containing 35 parts formamide and 65 parts purified water.

The hollow fibers were reacted with the PEO solution  
for ten hours without agitation. The PEO solution  
temperature was maintained at ambient temperature within  
30 the range from about 20°C to about 30°C. Upon removal from  
the PEO solution, the hollow fibers were rinsed with 100 ml  
of purified water to remove any unbound PEO bis(glycidyl  
ether).

EXAMPLE 20

Siloxane-coated hollow fibers onto which amine functionalities had been introduced according to the procedures of Example 1 were reacted with the PEO solution in accordance with the procedures of Example 19 with the exception that the hollow fibers were reacted with the PEO solution for 72 hours.

15 resulted in only slightly increased PEO concentration on the surface.

20 Siloxane-coated hollow fibers on which amine functionalities have been incorporated onto the siloxane surface according to the procedure of Example 9 were reacted with the PEO solution in accordance with the procedure of Example 19.

25 surface of the fiber was in the form of an ether  
functionality. It was assumed that all ether-type carbon  
atoms were due to PEO coupling.

30 Siloxane-coated fibers onto which amine functionalities had been introduced according to the procedure of Example 9 were reacted with the PEO solution in accordance with the procedure of Example 20.



1       ESCA analysis indicated that 22% of the carbon on the  
surface of the fiber was in the form of an ether function-  
ality. It was assumed that all ether-type carbon atoms  
were due to PEO coupling. This demonstrates that the  
5 additional reaction time did not result in an increased PEO  
concentration on the surface.

#### EXAMPLE 23

10       Polyethylene microporous hollow fibers with a siloxane  
coating onto which amine functionalities had been  
introduced according to the procedure of Example 1 were  
reacted with the PEO solution in accordance with the  
procedure of Example 19 with the exception that the PEO had  
a molecular weight of 600 at a concentration of 5%.

15       ESCA analysis indicated that 50% of the carbon on the  
surface of the fiber was in the form of an ether  
functionality. This demonstrates that higher efficiency  
coupling can be obtained using lower molecular weight PEO.

#### EXAMPLES 24-29

20       In Examples 24-29, amine-containing siloxane-coated  
hollow fibers prepared in accordance with Examples 2-4 and  
10-12, respectively, are reacted with the PEO solution  
according to the procedures set forth in Example 19.

25       Upon analysis, it is determined that 15-22% of the  
carbon on the surface is in the form of an ether  
functionality. It is assumed that all ether-type carbon  
atoms are due to PEO coupling. The higher the nitrogen  
incorporation, the higher the PEO coupling efficiency.

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EXAMPLES 30-34

In Examples 30-34, the siloxane-coated polymeric substrate incorporating the amine functionalities prepared according to the procedures of Examples 5-8 and 14, respectively, are reacted with the PEO solution in accordance with the procedures of Example 19.

Upon analysis, it is determined that 15-25% of the carbon on the surface of the hollow fibers is in the form of an ether functionality. It is assumed that all ether-type carbon atoms are due to PEO coupling.

EXAMPLES 35-40

In Examples 35-40, amine-containing siloxane-coated hollow fibers are prepared according to the procedures of Examples 2-4 and 10-12, respectively, are reacted with a PEO solution in accordance with the procedures set forth in Example 20.

Upon analysis, it is determined that 15-25% of the carbon on the surface of the hollow fibers is in the form of an ether functionality. It is assumed that all ether type carbon atoms are due to PEO coupling.

EXAMPLES 41-45

In Examples 41-45, siloxane-coated polymeric substrates onto which amine functionalities had been introduced according to the procedures of Examples 5-8 and 14, respectively, are reacted with the PEO solution described in, according to the procedures of Example 20.

Upon analysis, it is determined that 15-25% of the carbon on the surface is in the form of an ether functionality. It is assumed that all ether-type atoms are due to PEO coupling.

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EXAMPLE 46

Amine-containing siloxane-coated hollow fibers, prepared in accordance with the procedures of Example 1, are reacted with a PEO solution prepared by dissolving five grams of PEO bis(isocyanate) having an average molecular weight of 3500 into 100 ml of dry methylene chloride. The reaction is performed under a nitrogen atmosphere.

The hollow fibers are reacted with the PEO solution for ten hours without agitation. The PEO solution temperature is maintained at ambient temperature within the range of from about 20°C to about 30°C. Upon removal from the PEO solution, the hollow fibers are rinsed with 100 ml of methylene chloride to remove any unbound PEO bis(isocyanate).

Upon analysis, it is determined that 45% of the carbon atoms on the surface are attributed to PEO attached to the siloxane surface of the hollow fibers.

EXAMPLE 47

In Example 47, amine-containing siloxane-coated hollow fibers prepared in accordance with the procedures of Example 1, are treated with a solution containing poly(ethylene oxide) bis(isocyanate), which is prepared by dissolving three grams of PEO bis(isocyanate) having an average molecular weight of 3500 into 100 ml of dry methylene chloride. The reaction is performed under a nitrogen atmosphere.

The hollow fibers are reacted with the PEO solution for 72 hours without agitation. The PEO solution temperature is maintained at ambient temperature within the range of from about 20°C to about 30°C. Upon removal from this PEO solution, the hollow fibers were rinsed with 100

35

1 ml of methylene chloride to remove any unbounded PEO bis(isocyanate).

Upon analysis, it is determined that 40% of the carbon atoms on the surface are attributed to PEO attached to the surface of the hollow fibers.

#### EXAMPLES 48-54

In Examples 48-54, siloxane-coated hollow fibers on which amine functionalities had been introduced, prepared in accordance with the procedures of Examples 2-4 and 9-12, are reacted with a PEO solution according to the procedures set forth in Example 46.

Upon analysis, it is determined that 55% of the carbon atoms on the surface are attributed to PEO attached to the siloxane surface of the siloxane-coated hollow fibers.

#### EXAMPLES 55-61

In Examples 55-61, siloxane-coated hollow fibers on which amine functionalities had been introduced, prepared in accordance with the procedures of Examples 2-4 and 9-12, are reacted with a PEO solution according to the procedures set forth in Example 47.

Upon analysis, it is determined that 40-55% of the carbon atoms on the surface are attributed to PEO coupling to the siloxane surface of the siloxane-coated hollow fibers.

#### EXAMPLES 62-66

In Examples 62-66, a siloxane-coated polymeric substrate onto which amine functionalities have been incorporated according to the procedures set forth in Examples 5-8 and 14, respectively, are reacted with the PEO

1 solution described in, according to the procedures of,  
Example 46.

Upon analysis, it is determined that 40-55% of the  
carbon atoms on the surface are attributed to PEO coupling  
5 to the siloxane surface of the siloxane-coated substrate.

#### EXAMPLES 67-71

In Examples 67-71, a siloxane-coated polymeric  
substrate into which amine functionalities have been  
10 incorporated according to the procedures set forth in  
Examples 5-8 and 14, respectively, are reacted with the PEO  
solution described in, according to the procedures of,  
Example 47.

Upon analysis, it is determined that 40-55% of the  
15 carbon atoms on the surface are attributed to PEO  
attachment to the siloxane surface of the siloxane-coated  
substrate.

#### 5. PEO Reaction With Bioactive Molecules.

20 According to the present invention, the unbound end of  
the PEO is reacted with bioactive molecules to covalently  
bond those bioactive molecules to the PEO which is itself  
bonded to the polymer surface. An important preferred  
embodiment of the present invention is to bind a plurality  
25 of different bioactive molecules to the PEO linkages in  
order to result in a polymer surface having multifunctional  
thrombo-resistant properties.

Such bonding of a plurality of bioactive molecules to  
the PEO on the siloxane surface of a blood gas exchange  
30 device occurs when the device is placed in a solution  
containing a variety of bioactive molecules (referred to  
generically as a "PIE" solution; "PIE" is an acronym for  
Prosthetic Intimal Endothelium). One preferred formulation

- 1 of a PIE solution within the scope of the present invention  
is set forth in Table III.

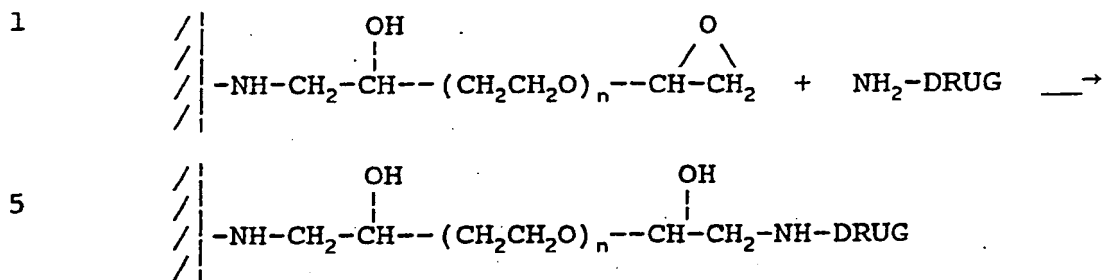
TABLE III

5	Heparin (80,000 USP units)	570 mg
	Urokinase powder (5% in formulation)	15 mg
	Ticlopidine	80 mg
	Plasmin Powder (activity 3-6 units/mg)	15 mg
	Tissue Plasminogen Activator (TPA)	15 mg
10	Prostaglandin E <sub>1</sub>	1 mg

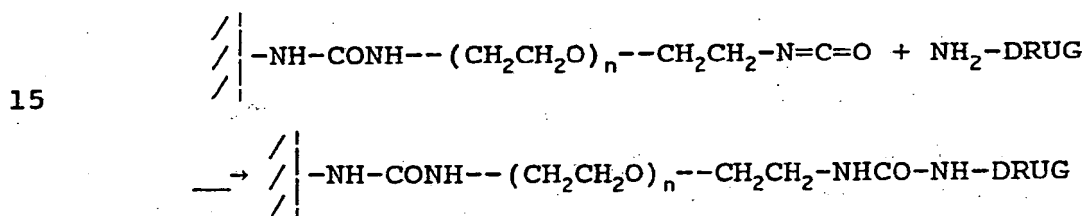
The PIE solution is prepared by dissolving heparin in 100 ml phosphate buffered saline (having a pH in the range of from about 7.1 to about 7.5 (preferably a pH of about 7.4) resulting in a concentration in the range from about 500 to about 1500 USP units per milliliter. Preferably, the heparin concentration is about 1000 USP units per milliliter. The remaining bioactives are added to the heparin solution in the amounts indicated in Table II.

20 The PEO/siloxane surface is soaked in the PIE solution for about 12 hours without agitation. The PIE solution is maintained at ambient temperature in the range from about 20°C to about 30°C. Upon removal from the solution, the surface is washed with purified water, air dried, and 25 sterilized with ethylene oxide.

It has been found that the bioactive molecules are coupled to the epoxide groups of the PEO chains through any primary amines available on the bioactive molecule. While the exact mechanism is not known, it is theorized that the 30 heparin, urokinase, plasmin, and TPA are coupled to the PEO as shown below.



"NH<sub>2</sub>-DRUG" refers to an amine-containing bioactive molecule. The bioactive molecules are coupled to isocyanate-terminated PEO chains through a similar mechanism shown below.



#### 20 D. Exemplary Embodiment of the Present Invention.

Further typical examples illustrating the method of preparing thrombo-resistant compositions within the scope of the present invention are given hereinbelow. These examples, as well as Examples 1-71, should be considered to be only illustrative of the present invention and not a complete identification of all embodiments of the present invention.

#### EXAMPLE 72

30 In Example 72, siloxane-coated hollow fibers onto which PEO chains have been introduced in accordance with the procedures of Examples 19, were reacted with a PIE solution containing various bioactive molecules.

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1       The PIE solution was prepared by obtaining eight (8)  
10-ml vials of heparin dissolved in phosphate buffered  
saline (pH 7.4) having a concentration of 1000 USP  
units/ml. Suitable heparin was obtained from Diosynth,  
5       Sigma, Organon, and Calbiochem. Other bioactive molecules  
were then dissolved into the heparin solution as follows:

15               15 mg urokinase powder (Sigma), 5% in formulation;  
                 80 mg ticlopidine (Syntex); and  
10               15 mg plasmin power (Sigma), with an activity from  
                 3-6 units/mg.

Additional phosphate buffered saline (pH 7.4) was added to  
give a total volume of 100 ml.

15       The PEO containing hollow fibers were immersed in the  
PIE solution for at least twelve (12) hours without  
agitation. The PIE solution was maintained at ambient  
temperature in the range from about 20°C to about 30°C.  
Upon removal from the PIE solution, the hollow fibers were  
20       rinsed ten (10) times with 100 ml of purified water to  
remove any unbound bioactive molecules. The hollow fibers  
were air dried and sterilized with ethylene oxide.

25       The surfaces were analyzed by ESCA and found to  
contain nitrogen and sulfur containing compounds. Analysis  
with trinitrobenzene sulfonic acid (TNBS) (an analytical  
technique for proteins) demonstrated measurable quantities  
of proteins on the surface (urokinase, plasmin, and TPA).  
Analysis using a solution depletion method with toluidine  
blue indicated heparin to be present in amounts similar or  
30       greater than those of other heparin-bound preparations  
reported in the literature.

Thrombogenicity tests were performed utilizing the  
procedures described in Mortensen et al., "A Practical  
Screening Test for Thrombogenicity of Intraarterial

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1 Catheters -- Preliminary Report," Artificial Organs, Vol.  
2, Supp., pp. 76-80, 1978, which is incorporated herein by  
reference. Thrombogenicity testing results have indicated  
5 that the bioactive molecules are present and active on the  
surface. Small bundles of treated hollow fibers were  
implanted into the carotid and femoral arteries of large  
dogs for a period of 30 minutes. The amount of adherent  
thrombus and that expelled from the artery following  
10 withdrawal of the bundle was weighed and found to be  
significantly less than that of the controls.

#### EXAMPLES 73

In Example 73, multifunctional thrombo-resistant  
hollow fibers were prepared in accordance with the  
15 procedure of Example 70, except that after the hollow  
fibers were removed from the PIE solution and rinsed with  
purified water, the hollow fibers were soaked in a one  
percent (1%) glutaraldehyde solution in a pH 7.4 phosphate  
buffer for one (1) hour. After removal from the  
20 glutaraldehyde solution, the hollow fibers were rinsed ten  
(10) times with 100 ml of a pH 7.4 phosphate buffer  
solution. The hollow fibers were then soaked in a 0.13 M  
glycine solution in a pH 7.4 phosphate buffer for 72 hours.  
Upon removal from the glycine solution, the hollow fibers  
25 were rinsed ten (10) times with 100 ml of purified water.  
The hollow fibers were air dried and sterilized with  
ethylene oxide.

The additional glutaraldehyde and glycine treatments  
increased the cross-linking of molecules on the substrate  
30 surface thereby enhancing the stability of the bioactive  
molecules. The surfaces were analyzed by ESCA according to  
the procedures of Example 72 and demonstrated measurable  
quantities of proteins and heparin on the surface.

1       Thrombogenicity testing according to the procedures  
given in detail in Example 72 have indicated that the  
bioactive molecules are present and active on the surface.  
No significant differences were noted between the fibers  
5       prepared according to the procedures of Example 72.

#### EXAMPLES 74-75

In Examples 74-75, siloxane-coated hollow fibers onto  
which PEO chains had been introduced in accordance with the  
10       procedures of Examples 19 and 21, respectively, were  
reacted with the PIE solution according to the procedures  
set forth in Example 70 except that the PIE solution  
contained the following bioactive substances in the  
indicated quantities: heparin (570 mg), urokinase (15 mg)  
15       and prostaglandin E<sub>1</sub> (1 mg).

The surfaces were analyzed according to the procedures  
described in Example 72 and demonstrated measurable  
quantities of proteins and heparin on the surface.

Thrombogenicity testing according to the procedures  
20       described in detail in Example 72 have indicated that the  
bioactive molecules are present and active on the surface.  
The thrombogenicity index of these samples were only  
slightly less than those of the samples prepared according  
to the procedures of Example 72.

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#### EXAMPLES 76-77

In Examples 76-77, siloxane-coated hollow fibers onto  
which PEO chains had been introduced in accordance with the  
procedures of Examples 19 and 21, respectively, are reacted  
30       with the PIE solution according to the procedures set forth  
in Example 72 except that the PIE solution contains the  
following bioactive substances in the indicated propor-  
tions: heparin, 570 mg; streptokinase, 15 mg; aspirin, 80  
mg; and sulfinpyrazone, 30 mg.

35

1       Thrombogenicity testing of these PIE coupled surfaces  
indicate that the bioactive molecules are present and  
active on the surface. The thrombogenicity index of the  
samples are slightly less than those of the samples  
5       prepared according to the procedures of Examples 72 and 74.

#### EXAMPLE 78

      Amine containing siloxane-coated hollow fibers  
prepared substantially in accordance with the procedures of  
10       Example 1 were placed in a PIE solution containing various  
bioactive molecules. The PIE solution was prepared  
according to the procedure described in Example 72.

      The amine-containing hollow fibers were immersed in  
the PIE solution for at least twelve (12) hours without  
15       agitation. The PIE solution was maintained at ambient  
temperature in the range from about 20°C to about 30°C.  
Upon removal from the PIE solution, the hollow fibers were  
rinsed ten times with 100 ml of purified water. The hollow  
fibers were air dried and sterilized with ethylene oxide.

20       The thrombogenicity tests indicated that the  
bioactives were present and active, even in the absence of  
PEO. ESCA, toluidine blue, and TNBS analysis indicated  
that an increased amount of the bioactives were coupled  
onto the surface. However, the higher amount of bioactives  
25       did not translate into greater biological activity.

#### EXAMPLES 79-80

      In Examples 79-80, siloxane-coated hollow fibers onto  
which PEO chains had been introduced in accordance with the  
30       procedures of Examples 19 and 21, respectively, are reacted  
with the PIE solution according to the procedures set forth

1 in Example 71, except that the PIE solution contains the  
following bioactive substances in the indicated  
proportions:

5 Heparin (80,000 USP units)  
570 mg  
Urokinase powder (Sigma, 5% in formulation)  
15 mg  
Ticlopidine (Syntex)  
10 80 mg  
Plasmin Powder (Sigma, activity 3-6 units/mg)  
15 mg  
Tissue Plasminogen Activator (TPA)  
15 mg  
15 Prostaglandin E<sub>1</sub>  
1 mg

Thrombogenicity testing of these PIE coupled surfaces  
indicate that the bioactive molecules are present and  
20 active on the surface. The thrombogenicity index of the  
samples are slightly greater than those of the samples  
prepared according the procedures of Examples 74 and 75.

Other drugs, not listed in Table II, are possible  
candidates for immobilization within the scope of the  
25 present invention. For example, drugs which have been used  
to passivate platelets include the following: sulfin-  
pyrazone (a prodrug), iloprost (a synthetic prostacyclin  
analogue) dipyramidole, aspirin, U-63557A, APS-306, and  
Prostacyclin (PGI<sub>2</sub>). Complement inhibitor candidates  
30 include the following drugs: FUT-175 (Nafamstet Mesilate),  
and p-Guanindinobenzate derivatives, and Chloroquine.  
Potential protein lysers (Fibrinolytics) include the  
following drugs: Streptokinase and APSAC. Finally, MD-805  
is a known thrombin inhibitor. Many of these drugs are  
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1 unsuitable for permanent covalent attachment due to their  
mechanism of action. However, if they were bound by a  
cleavable bond such as an amide bond, they could be  
released for local administration.

5 The drugs which are not suitable for the current  
preferred embodiment within the scope of the present  
invention include: sulfinpyrazone which must be  
metabolized before it is active; dipyramidole, which must  
enter into a platelet to be effective; and aspirin, which  
10 acetylates all other proteins and inactivates them.

Many of the foregoing drugs are still experimental and  
have not yet received Food and Drug Administration (FDA)  
approval for human use in the United States. Nevertheless,  
these drugs are given to illustrate the type of drugs which  
15 may be suitable for use within the scope of the present  
invention.

#### E. Summary

Although the above discussion has described a  
20 multifunctional thrombo-resistant coating for use with  
blood gas exchange devices, it will be appreciated that the  
thrombo-resistant coating may be adapted for use with other  
blood-contacting surfaces. Moreover, the principles  
described within the scope of the present invention may be  
25 used in connection with surfaces which initiate reactions  
similar to the blood-material incompatibility reactions.  
For instance, the principle of protein resistant surfaces  
achieved by terminally grafting PEO to the surface may be  
applied to contact lenses which are susceptible to protein  
30 deposit buildup. Other principles within the scope of this  
invention include the use of PEO coupled antibodies on  
chromatography supports. The PEO minimizes the nonspecific  
binding of protein while the bioactive antibodies are

1 active and capable of specifically isolating other  
molecules.

5 In summary, the multifunctional thrombo-resistant  
compositions and methods disclosed herein represent a  
significant departure from traditional thrombo-resistant  
coating techniques. The present invention counteracts a  
wide range of blood-material incompatibility reactions  
without inhibiting the gas permeability of the blood-  
contacting surface. This is accomplished by immobilizing  
10 various bioactive molecules which counteract blood material  
incompatibility reactions to the blood-contacting surface  
through individual poly(ethylene oxide) spacer chains.  
Because the bioactive molecules are tethered away from the  
blood-contacting surface, the molecules avoid problems of  
15 steric hindrance and possess an activity approaching the  
activity in solution. In addition, the bioactive molecules  
are covalently bound to the blood-contacting surface,  
thereby eliminating leaching of the bioactive molecules  
into the blood plasma and prolonging the effectiveness of  
20 the thrombo-resistant composition.

The present invention may be embodied in other  
specific forms without departing from its spirit or  
essential characteristics. The described embodiments are  
to be considered in all respects only as illustrative and  
25 not restrictive. The scope of the invention is, therefore,  
indicated by the appended claims rather than by the  
foregoing description. All changes which come within the  
meaning and range of equivalency of the claims are to be  
embraced within their scope.

30 What is claimed is:

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1           1.    A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood,  
the method comprising the steps of:

5           (a) obtaining a material having a siloxane  
surface onto which a plurality of amine functional  
groups have been bonded;

10           (b) reacting the amine functional groups on the  
siloxane surface with poly(ethylene oxide) chains  
terminated with functional groups capable of reacting  
with the amine functional groups on the siloxane  
surface, thereby resulting in a product having single  
poly(ethylene oxide) chains which are bonded to  
corresponding single amine functional groups;

15           (c) reacting the product of step (b) with a  
plurality of at least two different bioactive  
molecules capable of counteracting specific blood-  
material incompatibility reactions such that a single  
bioactive molecule is correspondingly coupled to a  
single poly(ethylene oxide) chain, thereby resulting  
20           in a siloxane surface to which are attached, by a  
poly(ethylene oxide) chain, a plurality of at least  
two different bioactive molecules which react with  
blood components which come in proximity to the  
siloxane surface of the material in order to resist  
25           blood-material incompatibility reactions.

30           2.    A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the step of obtaining a  
material having a siloxane surface onto which a plurality  
of amine functional groups have been bonded comprises the  
steps of:

35           introducing ammonia gas within a plasma chamber  
capable of performing plasma etching;

1           exposing the ammonia gas to a radio frequency of  
sufficient power to create a plasma; and  
          exposing the siloxane surface to the ammonia  
plasma for sufficient time to introduce amine  
5           functional groups onto the siloxane surface.

3. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 2, further comprising the step of  
10          obtaining a hollow fiber having a siloxane surface thereon.

4. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the step of obtaining a  
15          material having a siloxane surface onto which a plurality  
of amine functional groups have been bonded comprises the  
steps of:

          introducing a gaseous mixture of siloxane monomer  
and ammonia into a plasma chamber capable of  
20          performing plasma polymerization;

          exposing the gaseous mixture of siloxane monomer  
and ammonia to a radio frequency of sufficient power  
to create a plasma; and

          exposing the material to the plasma for  
25          sufficient time to deposit thereon a siloxane plasma  
polymer onto which amine functional groups have been  
bonded.

5. A method for producing a multifunctional thrombo-  
30          resistant coating for use on surfaces which contact blood  
as defined in claim 4, wherein the mixture of siloxane  
monomer and ammonia gas is introduced at a ratio of  
siloxane monomer to ammonia gas in the range from about  
10:1 about 1:10.

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6. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood as defined in claim 1, wherein the step of obtaining a material having a siloxane surface onto which a plurality of amine functional groups have been bonded comprises the steps of:

introducing a gaseous mixture of tetramethyldisiloxane and ammonia into a plasma chamber capable of performing plasma polymerization;

exposing the gaseous mixture of tetramethyldisiloxane and ammonia to a radio frequency of sufficient power to create a plasma; and

exposing the material to the plasma for sufficient time to deposit thereon a siloxane plasma polymer onto which amine functional groups have been bonded.

7. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood as defined in claim 10, wherein the mixture of siloxane monomer and ammonia gas is introduced into the plasma chamber in the range from about 3:1 to about 1:1 siloxane monomer to ammonia gas.

8. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood as defined in claim 10, further comprising the step of obtaining a microporous hollow fiber.

9. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood as defined in Claim 1, wherein the poly(ethylene oxide) chains terminated with functional groups capable of

35

1 reacting with the amine functional groups comprises  
poly(ethylene oxide) bis(glycidyl ether).

5 10. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in Claim 1, wherein the poly(ethylene oxide)  
chains terminated with functional groups capable of  
reacting with the amine functional groups comprises  
poly(ethylene oxide) bis(isocyanate).

10 11. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the poly(ethylene oxide)  
chains have a molecular weight in the range from about 1500  
15 to 6000.

20 12. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the product of step (b) is  
reacted with a solution having different bioactive  
molecules capable of resisting at least two of the  
following blood-material incompatibility reactions:  
extrinsic coagulation pathway activation, platelet  
destruction and injury, platelet adhesion, platelet  
25 aggregation, thrombus formation, and complement activation.

30 13. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the product of step (b) is  
reacted with a plurality of at least two different  
bioactive molecules selected from the group including  
heparin, urokinase, plasmin, and ticlopidine.

1           14. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the product of step (b) is  
reacted with a plurality of at least two different  
5 bioactive molecules selected from the group including  
heparin, urokinase, TPA, and prostaglandin E<sub>1</sub>.

          15. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
10 as defined in claim 1, wherein the product of step (b) is  
reacted with a plurality of at least two different  
bioactive molecules selected from the group including  
heparin, plasmin, and ticlopidine.

15           16. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 1, wherein the product of step (b) is  
reacted with a plurality of at least two different  
bioactive molecules selected from the group including  
20 heparin, urokinase, TPA, plasmin, prostaglandin E<sub>1</sub>, and  
ticlopidine.

          17. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood,  
25 the method comprising the steps of:

          (a) obtaining a material having a siloxane  
surface;

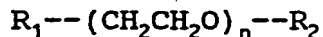
          (b) introducing ammonia gas within a plasma  
chamber capable of performing plasma etching;

30           (c) exposing the ammonia gas to a radio  
frequency of sufficient power to create a plasma;

          (d) exposing the siloxane surface to the ammonia  
plasma for sufficient time to introduce amine  
functional groups onto the siloxane surface, thereby  
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1 resulting in a product having a plurality of amine  
functional groups bonded onto the siloxane surface;

(e) reacting the product of step (d) with a  
solution having a plurality of poly(ethylene oxide)  
5 spacer chains, having the following general formula



wherein  $R_1$  and  $R_2$  are suitable functional groups  
capable of reacting with the amine functional groups  
on the siloxane surface; and

10 (f) reacting the product of step (e) with a  
plurality of at least two different bioactive  
molecules capable of counteracting specific blood-  
material incompatibility reactions such that a single  
bioactive molecule is correspondingly coupled to a  
15 single poly(ethylene oxide) spacer chain, thereby  
resulting in a siloxane surface to which are attached,  
by a poly(ethylene oxide) chain, a plurality of at  
least two different bioactive molecules which react  
with blood components which come in proximity to the  
20 surface of the material in order to resist blood-  
material incompatibility reactions.

18. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
25 as defined in claim 17, wherein  $R_1$  and  $R_2$  comprise glycidyl  
ether.

19. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
30 as defined in claim 17, wherein  $R_1$  and  $R_2$  comprise  
isocyanate.

20. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
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1 as defined in claim 17, wherein the product of step (e) is  
reacted with a plurality of at least two different  
bioactive molecules selected from the group including  
5 heparin, urokinase, plasmin, and ticlopidine.

21. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 17, wherein the product of step (e) is  
10 reacted with a plurality of at least two different  
bioactive molecules selected from the group including  
heparin, urokinase, plasmin, ticlopidine, TPA, and  
prostaglandin E<sub>1</sub>.

22. A method for producing a multifunctional thrombo-  
15 resistant coating for use on surfaces which contact blood,  
the method comprising the steps of:

(a) introducing a gaseous mixture of siloxane  
monomer and ammonia into a plasma chamber capable of  
performing plasma polymerization;

20 (b) exposing the gaseous mixture of siloxane  
monomer and ammonia to a radio frequency of sufficient  
power to create a plasma;

(c) exposing a material to the plasma for  
sufficient time to deposit onto the surface of the  
25 material a siloxane plasma polymer onto which amine  
functional groups have been bound;

(d) reacting the product of step (c) with a  
solution having a plurality of poly(ethylene oxide)  
spacer chains, having the following general formula

30 
$$R_1--(CH_2CH_2O)_n--R_2$$

wherein R<sub>1</sub> and R<sub>2</sub> are suitable functional groups  
capable of reacting with the amine functional groups  
on the siloxane surface; and

35

1 (e) reacting the product of step (d) with a  
plurality of at least two different bioactive  
molecules capable of counteracting specific blood-  
material incompatibility reactions such that a single  
5 bioactive molecule is correspondingly coupled to a  
single poly(ethylene oxide) spacer chain, thereby  
resulting in a siloxane surface to which are attached,  
by a poly(ethylene oxide) chain, a plurality of at  
least two different bioactive molecules which react  
10 with blood components which come in proximity to the  
surface of the material in order to resist blood-  
material incompatibility reactions.

23. A method for producing a multifunctional thrombo-  
15 resistant coating for use on surfaces which contact blood  
as defined in claim 22, wherein  $R_1$  and  $R_2$  comprise glycidyl  
ether.

24. A method for producing a multifunctional thrombo-  
20 resistant coating for use on surfaces which contact blood  
as defined in claim 22, wherein  $R_1$  and  $R_2$  comprise  
isocyanate.

25. A method for producing a multifunctional thrombo-  
25 resistant coating for use on surfaces which contact blood  
as defined in claim 22, wherein the mixture of siloxane  
monomer and ammonia gas is introduced into the plasma  
chamber at a ratio of siloxane monomer to ammonia gas in  
the range of from about 10:1 to about 1:10.

26. A method for producing a multifunctional thrombo-  
resistant coating for use on surfaces which contact blood  
as defined in claim 22, wherein the introducing step  
comprises introducing a gaseous mixture of  
35

1 tetramethyldisiloxane and ammonia into a plasma chamber.

27. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood  
5 as defined in claim 22, further comprising the step of covalently bonding a plurality of carbonyl functional groups onto the siloxane surface.

28. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood  
10 as defined in claim 22, further comprising the step of covalently bonding a plurality of hydroxyl functional groups onto the siloxane surface.

29. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood  
15 as defined in claim 22, wherein the product of step (d) is reacted with a plurality of at least two different bioactive molecules selected from the group including  
20 heparin, urokinase, plasmin, and ticlopidine.

30. A method for producing a multifunctional thrombo-resistant coating for use on surfaces which contact blood  
25 as defined in claim 22, wherein the product of step (d) is reacted with a plurality of at least two different bioactive molecules selected from the group including heparin, urokinase, plasmin, ticlopidine, TPA, and prostaglandin E<sub>1</sub>.

31. A multifunctional thrombo-resistant composition  
30 for use on surfaces which contact blood comprising a material having a siloxane surface onto which a plurality of at least two different bioactive molecules are covalently bonded, said bioactive molecules counteracting  
35

1 specific blood-material incompatibility reactions when the  
blood comes into proximity of the surface of the material.

5 32. A multifunctional thrombo-resistant composition  
for use on surfaces which contact blood as defined in claim  
31 further comprising a plurality of poly(ethylene oxide)  
chains covalently bonded to the bioactive molecules and  
covalently bonded to the siloxane surface such that a  
10 single bioactive molecule is correspondingly coupled to a  
single poly(ethylene oxide) chain which in turn is bonded  
to the siloxane surface.

15 33. A multifunctional thrombo-resistant composition  
for use on surfaces which contact blood as defined in claim  
31, wherein the plurality of different bioactive molecules  
are capable of resisting at least two of the following  
blood material incompatibility reactions: extrinsic  
coagulation pathway activation, platelet destruction and  
injury, platelet adhesion, platelet aggregation, thrombus  
20 formation, and complement activation.

25 34. A multifunctional thrombo-resistant composition  
for use on surfaces which contact blood as defined in claim  
31, wherein the plurality of different bioactive molecules  
are selected from the group including heparin, urokinase,  
plasmin, ticlopine, TPA, and prostaglandin E<sub>1</sub>.

30 35. A multifunctional thrombo-resistant composition  
for use on surfaces which contact blood, the composition  
being made by a process comprising the steps of:

(a) obtaining a material having a siloxane  
surface onto which a plurality of amine functional  
groups have been bonded;

35

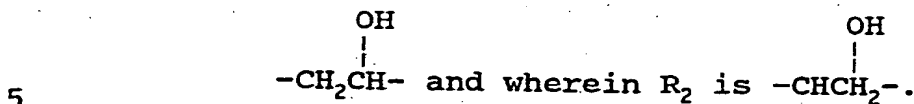


1 (b) reacting the amine functional groups on the  
siloxane surface with poly(ethylene oxide) chains  
terminated with functional groups capable of reacting  
with the amine functional groups on the siloxane  
5 surface such that a single poly(ethylene oxide) chain  
is bonded to a corresponding single amine functional  
group; and

(c) reacting the product of step (b) with a  
plurality of at least two different bioactive  
10 molecules capable of counteracting specific blood-  
material incompatibility reactions such that a single  
bioactive molecule is covalently bonded to a single  
poly(ethylene oxide) chain, thereby resulting in a  
siloxane surface to which are attached, by a  
15 poly(ethylene oxide) chain, a plurality of at least  
two different bioactive molecules which react with  
blood components which come in proximity to the  
siloxane surface of the material in order to resist  
blood-material incompatibility reactions.

20 36. A multifunctional thrombo-resistant composition  
comprising a plurality of compounds having the formula  
 $X-NH-R_1--(CH_2CH_2O)_n--R_2-Y$  and  $X-NH-R_1--(CH_2CH_2O)_n--R_2-Z$   
wherein X is a siloxane surface; and wherein  $R_1$  and  $R_2$  are  
25 the residue resulting from a reaction between a  
poly(ethylene oxide) terminal group capable of reacting  
with an amine and capable of reacting with a bioactive  
molecule, respectively; and wherein Y and Z are different  
bioactive molecules capable of counteracting a specific  
30 blood material incompatibility reaction.

1           37. A multifunctional thrombo-resistant composition  
as defined in claim 36, wherein R<sub>1</sub> is



          38. A multifunctional thrombo-resistant composition  
as defined in claim 36, wherein R<sub>1</sub> is -CONH- and wherein R<sub>2</sub>  
is -NHCO-.

10           39. A multifunctional thrombo-resistant coating as  
defined in claim 36, wherein X or Y is heparin, urokinase,  
15 tissue plasminogen activator, or plasmin.

          40. A multifunctional thrombo-resistant coating as  
defined in claim 36, wherein X or Y is heparin,  
20 ticlopidine, or urokinase.

          41. A multifunctional thrombo-resistant coating as  
defined in claim 36, wherein X or Y is heparin,  
25 prostaglandin E<sub>1</sub>, plasmin, urokinase, or tissue plasminogen  
activator.

          42. A multifunctional thrombo-resistant coating as  
30 defined in claim 36, wherein X or Y is heparin,  
ticlopidine, plasmin, urokinase, tissue plasminogen  
activator, or FUT-175.

35

1           43. A multifunctional thrombo-resistant coating as  
defined in claim 36, wherein X or Y is capable of resisting  
either extrinsic coagulation pathway activation, platelet  
5       destruction and injury, platelet adhesion, platelet  
aggregation, thrombus formation, or complement activation.

10           44. A gas permeable membrane for effecting  
extrapulmonary blood gas exchange, the membrane comprising  
a gas permeable substrate which is coated with a polyfunc-  
tional thrombo-resistant composition comprising a siloxane  
15       surface onto which a plurality of at least two different  
bioactive molecules are covalently bonded, said bioactive  
molecules being capable of counteracting specific blood-  
material incompatibility reactions.

20           45. An apparatus for effecting extrapulmonary blood  
gas exchange comprising:

          a plurality of gas permeable tubes, each tube  
having a first end and a second end, said gas  
25       permeable tubes being coated with a multifunctional  
thrombo-resistant composition comprising a siloxane  
surface onto which a plurality of at least two  
different bioactive molecules are covalently bonded,  
30       said bioactive molecules being capable of  
counteracting specific blood-material incompatibility  
reactions;

35

1 tube means comprising a first lumen and a second  
lumen, one of said first and second lumens extending  
between the first and second ends of the gas permeable  
5 tubes and the first lumen terminating adjacent to the  
first ends of the gas permeable tubes and the second  
lumen terminating adjacent to the second ends of the  
gas permeable tubes;

10 means for introducing oxygen from the first lumen  
into the first end of the gas permeable tubes whereby  
blood in contact with the gas permeable tubes receives  
oxygen from the gas permeable tubes and releases  
15 carbon dioxide gas to the gas permeable tubes; and

means for collecting carbon dioxide at the second  
ends of the gas permeable tubes and introducing said  
20 carbon dioxide into the second lumen for removal  
therethrough.

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(21) International Application Number: PCT/US89/01853 (22) International Filing Date: 1 May 1989 (01.05.89) (30) Priority data: 215,014 5 July 1988 (05.07.88) US (71) Applicant: CARDIOPULMONICS, INC. [US/US]; 419 Wakara Way, Suite 110, University of Utah Research Park, Salt Lake City, UT 84108 (US). (72) Inventors: WINTERS, Suzanne ; 637 2nd Avenue, Salt Lake City, UT 84103 (US). SOLEN, Kenneth, A. ; 536 East 500 South, Orem, UT 84058 (US). SANDERS, Clifton, G. ; 633 East 200 South, Apt. 1, Salt Lake City, UT 84102 (US). MORTENSEN, J., D. ; 10600 Dimple Dell Road, Sandy, UT 84092 (US). BERRY, Gaylord ; 1896 Vine Street, Salt Lake City, UT 84121 (US).		(74) Agents: NYDEGGER, Rick, D. et al.; Workman, Nydegger & Jensen, 1000 Eagle Gate Tower, 60 East South Temple, Salt Lake City, UT 84111 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (Utility model), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 22 February 1990 (22.02.90)	
(54) Title: MULTIFUNCTIONAL THROMBO-RESISTANT COATINGS AND METHODS OF MANUFACTURE			
(57) Abstract <p>The present invention is directed to multifunctional thrombo-resistant coatings for use with biomedical devices and implants, such as a coating which includes a siloxane surface onto which a plurality of amine functional groups have been bonded. Covalently bonded to the amine functional groups are a plurality of poly(ethylene oxide) chains, such that a single poly(ethylene oxide) chain is bonded to a single amine functional group. A plurality of different bioactive molecules, designed to counteract specific blood-material incompatibility reactions, are covalently bonded to poly(ethylene oxide) chains, such that a single bioactive molecule is coupled to a single polyethylene oxide chains. The methods of manufacturing the present invention include preparing a material having a siloxane surface onto which a plurality of amine functional groups have been bonded. This is achieved by plasma etching with ammonia gas or by plasma polymerization of a siloxane monomer in the presence of ammonia gas. The amine-containing siloxane surface is reacted with poly(ethylene oxide) chains terminated with functional groups capable of reacting with the amine groups on the siloxane surface. The material is then reacted with a plurality of different bioactive molecules which counteract the specific blood-material incompatibility reactions, such that a single bioactive molecule is coupled to a single poly(ethylene oxide) chain. The resulting siloxane surface contains a plurality of different bioactive molecules capable of reacting with blood components which come in proximity to the siloxane surface in order to resist blood-material incompatibility reactions.</p>			

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# INTERNATIONAL SEARCH REPORT

PCT/US89/01853

International Application No.

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(4): A61M 1/14		
U.S. Cl.: 422/48; 428/447,450; 210/321.62,500.24,500.25		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
U.S.	422/48; 428/447,450; 210/321.62,500.24,500.25	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the ...		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b>		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
Y	US, A, 3,969,240 (KOLOBOW ET AL) 13 July 1976	1-45
Y	US, A, 4,093,515 (KOLOBOW) 06 June 1978	1-45
Y	US, A, 4,170,559 (KROPLINSKI ET AL) 09 October 1979	1-45
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Y P	US, A, 4,770,852 (TAKAHARA ET AL) 13 September 1988	1-45
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